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Altered Thyroid Axis Function in Lewis Rats with Genetically Defective Hypothalamic CRH/VP Neurosecretory Cells

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Contents

Introduction	1
Materials and Methods	1
Results	2
Discussion	3
Acknowledgments	4
References	4

Abstract. Lewis rats display a hyporesponsive hypothalamo-pituitary-adrenocortical (HPA) axis, overproduction of cytokines, and susceptibility to inflammatory disease. The Lewis corticotropin-releasing hormone (CRH) neurosecretory system contains normal numbers of vasopressin (VP) deficient axon varicosities but abnormally sparse vasopressin-containing varicosities in the external zone of the median eminence, compared to the normoresponsive Sprague Dawley (SD), Wistar, and Fischer 344 strains. Since vasopressin may act as a thyrotropin-releasing factor, we hypothesized that thyroid axis responsivity may be altered in Lewis rats. T_3 , T_4 , and the thyroid-stimulating hormone (TSH) were measured by radioimmunoassay, and free T_4 by equilibrium dialysis in adult male Lewis and SD rats. Exposure to cold (5°C) for 1 h induced significant increases in T_3 , T_4 , and TSH levels in Lewis rats but not in SD rats. Ninety min of insulin-induced hypoglycemia (1 IU/kg, ip) caused a significant T_3 increase in Lewis rats and a significant T_4 increase in SD rats. Two h after intraperitoneal administration of lipopolysaccharide (LPS; (0.25 or 0.75 mg/kg)), T_4 levels fell significantly in Lewis rats but not in SD rats. TSH decreases were significant in Lewis rats after 0.75 mg/kg LPS and in SD rats after 0.25 mg/kg. Baseline hormone levels were generally higher in Lewis rats; the differences were significant for T_3 and T_4 in the insulin experiments and for T_3 , T_4 , and free T_4 in the LPS experiments. The data suggest that reduced inhibition from the adrenocortical axis in Lewis rats leads to hyperresponsivity of the thyroid axis to cold and greater LPS-induced decreases in T_4 levels, probably due to an exaggerated inhibitory cytokine response.

Introduction

Lewis rats display blunted adrenocorticotrophic hormone (ACTH) and corticosterone responses to stimuli and lower 24-h levels of glucocorticoids than other standard laboratory strains [1-7]. We recently showed that the parvocellular corticotropin-releasing hormone (CRH) of the neurosecretory system in Lewis rats is defective in terms of vasopressin (VP) content [8], in contrast to the magnocellular system that contains abnormally high levels of VP [9]. Preliminary results indicate that Lewis rats exhibit abnormally high mortality after whole-body gamma irradiation (unpublished) and low thyroid hormone levels during radiation-induced sepsis, compared to Sprague Dawley (SD) rats (unpublished). Since VP may regulate the release of the thyroid-stimulating hormone (TSH) from the anterior pituitary [10, 11], we tested the hypothesis that thyroid regulation in Lewis rats is abnormal. Cold and insulin-induced hypoglycemia were used to stimulate the thyroid axis. Bacterial lipopolysaccharide (LPS) was used as a model of sepsis to inhibit the thyroid axis. Lewis rats were compared to SD

rats, a standard rat strain with a normoresponsive hypothalamo-pituitary-adrenocortical (HPA) axis [1, 8].

Materials and Methods

Male SD and Lewis rats, 250 ± 25 g in body weight, were purchased from the National Cancer Institute, kept on a 12-h light-dark schedule, and given food and water *ad libitum*, except during insulin experiments when food was withdrawn 18 h before insulin injection. For each of the three treatments (cold, insulin, and LPS), reported values are means of pooled results from two experiments.

In the cold experiments, animals were briefly immersed in room temperature water to reduce the insulating properties of their fur and were then placed in standard plastic rat-housing boxes (with no bedding) in a cold room (5°C) for one h. Control animals were not immersed and were kept in an adjacent room at room temperature (24°C) in their housing boxes (with bedding).

In the insulin experiments, animals received ip injections of 1 IU/kg recombinant human insulin (Lilly) or vehicle (phosphate-buffered saline, PBS). Blood was collected 90 min after the injection. Plasma glucose was measured on a Kodak Ektachem-700 analyzer.

In the LPS experiments, animals received ip injections of 0.25 or 0.75 mg/kg *E. coli* 0127:B8 LPS (DIFCO, Detroit, MI) or vehicle (PBS). Blood was collected 120 min after injection.

Trunk blood was collected, and plasma was prepared for radioimmunoassay of T_3 , T_4 , TSH, and ACTH as described previously [8, 12]. Free T_4 was assayed by equilibrium dialysis using kits from the Nichols Institute of Diagnostics, San Juan Capistrano, CA.

Significant differences are reported at p values of less than 0.05 and were obtained using analysis of variance and Fisher's protected least significant difference test.

Results

As expected, baseline levels of ACTH were similar in Lewis and SD rats, and increases after cold exposure, insulin, treatment or LPS treatment were significantly higher in SD rats (not shown).

The effects of cold exposure on thyroid hormone levels are shown in table 1. One h of cold exposure resulted in significant elevations of plasma total T_3 and total T_4 in Lewis rats but not in SD rats. TSH was also significantly elevated in Lewis rats but not in SD rats. Free T_4 was not significantly elevated in either strain (table 1).

Insulin-induced hypoglycemia in fasting animals was associated with significant elevations of total T_3 in Lewis rats but not in SD rats (table 1), similar to observations after cold exposure. However, total T_4 was significantly elevated in SD rats but not in Lewis rats after this treatment. The treatment caused no significant changes in free T_4 . TSH levels were significantly elevated in both strains. Control levels

Table 1. Plasma hormone levels after cold treatment, insulin-induced hypoglycemia, and LPS treatment in Sprague Dawley and Lewis rats.

Group	T_3 (ng/ml)	N	T_4 (ng/ml)	N	Free T_4 (pmol/L)	N	TSH (ng/ml)	N
<u>Cold treatment</u>								
SD-Control	0.673 ± .038	12	4.52 ± .17	7	30.1 ± 1.0	4	1.23 ± .20	4
SD-Cold	0.753 ± .050	20	4.83 ± .15	16	32.9 ± 4.4	7	1.19 ± .15	7
LEW-Control	0.762 ± .035	12	4.96 ± .21	8	35.9 ± 2.4	4	0.52 ± .07 [†]	4
LEW-Cold	0.949 ± .050*	20	5.48 ± .17*	16	44.3 ± 3.8	8	1.45 ± .15*	8
<u>Insulin treatment</u>								
SD-Control	0.575 ± .025	15	3.78 ± .24	15	34.0 ± 2.8	6	0.381 ± .046	14
SD-Insulin	0.578 ± .021	17	4.66 ± .23*	17	32.3 ± 1.9	7	0.512 ± .034*	7
LEW-Control	0.662 ± .029 [†]	12	5.20 ± .16 [†]	12	39.6 ± 3.4	6	0.389 ± .034	12
LEW-Insulin	0.752 ± .033*	13	5.69 ± .18	13	40.5 ± 2.9	7	0.547 ± .053*	13
<u>LPS treatment</u>								
SD-Control	0.627 ± .015	14	5.03 ± .20	8	34.0 ± 1.7	4	0.436 ± .044	8
SD-0.25 mg/kg	0.508 ± .024*	17	4.63 ± .20	8	40.9 ± 2.3	4	0.297 ± .014*	8
SD-0.75 mg/kg	0.462 ± .022*	18	4.62 ± .20	9	ND		0.350 ± .055	9
LEW-Control	0.762 ± .067 [†]	8	6.20 ± .18 [†]	8	47.0 ± 3.6 [†]	4	0.487 ± .044	7
LEW-0.25 mg/kg	0.606 ± .037*	8	4.78 ± .22*	8	49.0 ± 5.1	4	0.394 ± .070	8
LEW-0.75 mg/kg	0.612 ± .041*	9	5.20 ± .29*	9	ND		0.309 ± .016*	9

Values: mean ± SEM. N, number of animals in group. * Significantly different from control, p 0.05, one-way ANOVA. [†] Significant difference between SD-Control and LEW-Control. ND, not done.

of T_3 and T_4 were significantly higher in Lewis rats than in SD rats. Plasma glucose was slightly but significantly lower in Lewis rats than in SD rats in baseline conditions (100 ± 4.7 vs. 107 ± 5.8 mg/dl). The insulin-induced decrease in glucose was somewhat blunted in Lewis rats, resulting in final levels that were significantly higher than in SD rats (48.5 ± 4.2 vs. 39.8 ± 4.7 mg/dl). The percent decreases were 52% ($\pm 10\%$) and 63% ($\pm 16\%$) for Lewis and SD rats, respectively (standard errors of percent changes calculated by error propagation).

After LPS injections, levels of total T_4 were significantly decreased in Lewis rats but not in SD rats (table 1). Total T_3 levels were significantly decreased in both strains. Free T_4 levels were not affected. TSH levels were significantly decreased after doses of 0.25 mg/kg in SD rats and 0.75 mg/kg in Lewis rats. Control levels of T_3 , T_4 , and free T_4 were significantly higher in Lewis rats than in SD rats.

Discussion

Previous studies have shown that the adrenocortical axis in Lewis rats is hyporesponsive compared to SD, Fischer 344, and Wistar rats [1, 2]. Although there appears to be a defect in pituitary corticotropes in Lewis rats [13], hyporesponsiveness of the axis is due at least in part to relatively low expression of CRH [2] and VP in the hypothalamic neurosecretory system that regulates corticotropes [8]. It is also possible that there is a defect in the secretory function of these neurosecretory axons in Lewis rats [14]. *In vitro* studies demonstrated defective secretion of CRH from the hypothalami of Lewis rats [6]. In conscious rats, ip injection of LPS induced subnormal release of CRH and VP from parvocellular axon terminals in Lewis rats compared to SD rats, primarily due to significantly lower baseline levels of stored CRH and VP [8].

We were led to test the hypothesis that the thyroid axis is abnormal in Lewis rats because of evidence that VP may regulate secretion of TSH from thyrotrope cells in the anterior lobe of the pituitary. When pituitary cells were incubated with VP *in vitro*, there was a significant and dose-related release of TSH, but not of prolactin, growth hormone, follicle-

stimulating hormone, or luteinizing hormone, suggesting that VP has thyrotropin-releasing hormone-like activity [10]. The presence of VP receptors on thyrotrope cells also supports the notion that VP regulates TSH release [11]. Since Lewis rats possessed abnormally few VP-containing secretory vesicles in the neurosecretory system that regulates the anterior pituitary, we predicted thyroid axis function would be lower in this strain.

Contrary to our expectations, we found that baseline levels of thyroid hormones were generally higher in Lewis rats than in SD rats, and that changes in thyroid hormone levels after cold exposure were greater in Lewis rats. These data are reminiscent of the study of Fujimoto and Hedge [15] who found that a mutant strain of rat (Brattleboro) with a genetic deficiency of VP synthesis displayed abnormally high resting TSH levels. However, that strain is completely lacking in central VP, including the magnocellular neurosecretory system that regulates fluid homeostasis and electrolyte balance. The multiple endocrine abnormalities in that strain could affect thyroid axis function [10].

Our results suggest that baseline thyroid function in Lewis rats is enhanced due to reduced inhibition from the HPA axis. Activity levels of the thyroid and HPA axes are inversely correlated in many situations [16-25]. In the study by Lumpkin et al. [10], VP injected into the third ventricle of conscious rats caused specific decreases in plasma TSH levels, leading the authors to suggest that VP may act in a negative ultrashort feedback loop. The synapses observed between parvocellular CRH and TRH neurosecretory cells could mediate mutual inhibition between the two axes [26]. Inhibition of the thyroid axis by glucocorticoids has been demonstrated [16, 18, 27-31]. Glucocorticoids inhibit hypothalamic TRH expression [31], and may act at other levels of the thyroid axis [32].

It has been proposed that increased activity in the thyroid axis can result in inhibition of the adrenocortical axis [24]. One possible "corticotropin release-inhibiting factor" (CRIF) is the preprothyrotropin-releasing hormone-(178-199) [24], although Nicholson and Orth failed to confirm this result [33]. However, hyperthyroidism in Lewis rats probably is

not a major cause of the hyporesponsivity of the HPA axis. The abnormalities in adrenocortical function in Lewis rats are more consistent and of higher magnitude than the thyroid abnormalities. Therefore, it is likely that the primary defect in Lewis rats is hypoactivity of the HPA axis leading to changes in thyroid regulation.

It is well known that cytokines inhibit the thyroid axis [34-39], partly due to suppression of TRH gene expression in the hypothalamus [39]. This inhibition may account for the depression of thyroid function commonly observed in severely sick patients whose illnesses do not primarily affect the thyroid axis. The Lewis rat is characterized by exaggerated cytokine responses to stimuli, presumably due to defective inhibition of cytokine expression by the hypoactive HPA axis [7]. Higher cytokine release after LPS challenge in Lewis rats would explain the greater decrease in thyroid hormone levels seen in this strain compared to SD rats.

In spite of the changes in levels of T_3 , T_4 , and TSH induced by the challenges, no significant changes in free T_4 levels were observed, suggesting that changes in free T_4 levels were blunted by binding proteins. The only significant difference observed between any of the groups in terms of free T_4 levels was between control groups of the two strains in the LPS experiments. This difference was consistent with our overall observation of higher resting levels of thyroid hormones in Lewis rats.

In summary, the present findings demonstrate altered thyroid responses in Lewis rats compared to SD rats. The Lewis strain may provide a model for clinical syndromes associated with decreased inhibition of the thyroid axis by the HPA axis and increased inhibition of the thyroid axis by cytokines.

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13. ABSTRACT (Maximum 200 words) <p>Lewis rats display a hyporesponsive hypothalamo-pituitary-adrenocortical (HPA) axis, overproduction of cytokines, and susceptibility to inflammatory disease. The Lewis corticotropin-releasing hormone (CRH) neurosecretory system contains normal numbers of vasopressin (VP) deficient axon varicosities but abnormally sparse vasopressin-containing varicosities in the external zone of the median eminence, compared to the normoresponsive Sprague Dawley (SD), Wistar, and Fischer 344 strains. Since vasopressin may act as a thyrotropin-releasing factor, we hypothesized that thyroid axis responsivity may be altered in Lewis rats. T₃, T₄, and the thyroid-stimulating hormone (TSH) were measured by radioimmunoassay, and free T₄ by equilibrium dialysis in adult male Lewis and SD rats. Exposure to cold (5°C) for 1 h induced significant increases in T₃, T₄, and TSH levels in Lewis rats but not in SD rats. Ninety min of insulin-induced hypoglycemia (1 IU/kg, ip) caused a significant T₃ increase in Lewis rats and a significant T₄ increase in SD rats. Two h after intraponeal administration of lipopolysaccharide (LPS; (0.25 or 0.75 mg/kg)), T₄ levels fell significantly in Lewis rats but not in SD rats. TSH decreases were significant in Lewis rats after 0.75 mg/kg LPS and in SD rats after 0.25 mg/kg. Baseline hormone levels were generally higher in Lewis rats; the differences were significant for T₃ and T₄ in the insulin experiments and for T₃, T₄, and free T₄ in the LPS experiments. The data suggest that reduced inhibition from the adrenocortical axis in Lewis rats leads to hyperresponsivity of the thyroid axis to cold and greater LPS-induced decreases in T₄ levels, probably due to an exaggerated inhibitory cytokine response.</p>				
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